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Effect of α-Cyclodextrin on Fluorouracil

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Abstract:
α-cyclodextrin (α-CD) contain six glucopyronose units with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity in the center. α-cyclodextrin combine with fluorouracil to form an inclusion complex. Spectral characteristics of fluorouracil have been studied in α-cyclodextrin. The inclusion complex of α-cyclodextrin with fluorouracil is investigated by UV-visible and fluorimetry spectroscopy. The absorption maxima of fluorouracil appear at 265.6 nm. Upon increasing the concentration of α-CD the absorption maxima is blue shifted from 265.6 nm to 246.4 nm. The absorption intensity increases with increasing the concentration of α-CD. In emission spectra it is red shifted from 385 nm to 413 nm. The emission intensity is increased by the addition of α-CD. The association constant (K) of the Benesi-Hildebrand plot confirmed that 1:1 stoichiometric ratio was present in the inclusion complex. Phase solubility studies were carried out.

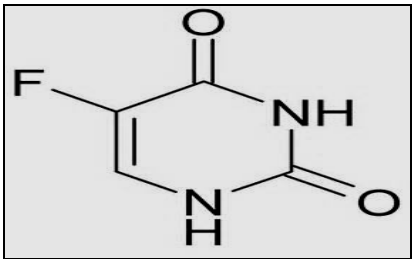
1. Introduction

Cyclodextrins are cyclic oligosaccharides containing a minimum of six D - (+) -glycopyranose units attached by α-1, 4-linkages produced by the action of the cyclodextrin - trans- glycosidase enzyme on a medium containing starch. It has a hydrophobic central cavity and a hydrophilic outer surface[(1)] .Cds have been found to be very useful in enhancing the solubility of poorly water soluble drugs owing to the formation of inclusion complex of the drug in its hydrophobic cavity[(2-6)].

Cyclodextrins have well known effects on drugs solubility and dissolution, bioavailability, safety and stability. There are various factors is influencing inclusion complex formation and an understanding of these factors is necessary for proper handling of these versatile materials. Some important considerations in selecting CDs in drug formulation are their commercial availability, regulatory status, and patent status. CDs are expected to solve many problems associated with the delivery of different novel drugs through different delivery routes[(7)]. Flurouracil is a drug that is a pyrimidine analog which is used in the treatment of cancer.It belongs to the family of drugs called the antimetabolites[(8)].

- IUPAC name of Fluorouracil is -5-fluro pyrimidine 2, 4 – dione
- Molecular formula of Fluorouracil is – $C_4H_3FN_2O_2$
- Molecular mass of Fluorouracil is – 130.077g/mol-1
- Trade names of Fluorouracil is – Adrucil, Carac, Efudex

2. Structure of Fluorouracil



3. Experimental

Absorption spectral measurements carried out with a Double beam Spectrophotometer Smart2203.Fluorescence measurements carried out with Jasco-Spectroflurometer FP - 8200, α-Cyclodextrin, Methanol were obtained from Ponmani Chemical glass agencies. Deionized water was used for the preparation of aqueous solutions. All solvents used were of the highest grade commercially available. The solutions were prepared just before taking the measurements.

3.1. A- Cyclodextrin Solution Preparation

The solutions of stock Fluorouracil transferred in to10ml volumetric flasks containing 0.002, 0.004,… 0.01 mol dm^{-3} α-CD solution. The mixed solution was diluted to 10ml with deionized water and shaken thoroughly. The absorption and Fluorescence spectra were recorded.

3.2. Phase Solubility Studies

The phase solubility studies were performed according to the method reported by Higuchi and Connors. Phase solubility permits the evaluation of the affinity between α-CD and fluorouracil in water. Fluorouracil in amounts that exceed its solubility was taken in to vials to which were added 15ml of distilled water containing various concentrations of α-CD.These flasks were sealed and shaken at 20^{o}C for 5 days. This amount of time is considered sufficient to reach equilibrium. Subsequently the aliquots where withdrawn using a stringe 1 hour intervals and samples where filtered immediately through whatman no 1 filter paper and approximately diluted a portion of the sample was analyzed by UV –Visible Spectrphotometer. The solubility experiments were conducted in triplicate[9].

4. Results and Discussion

SI. No	α-CD concentration	λabs(nm)	intensity	λflu(nm)	intensity
1	0	265.6	1.420	385	3398.41
2	0.002	263.8	1.803	400	3738.94
3	0.004	260.2	1.812	402	3833.93
4	0.006	252.0	1.873	406	4243.74
5	0.008	249.6	1.896	408	4285.91
6	0.01	246.4	2.466	413	4599.87

Table 1: Absorption and Fluorescence maxima (nm) of Fluorouracil at different concentration of α-CD

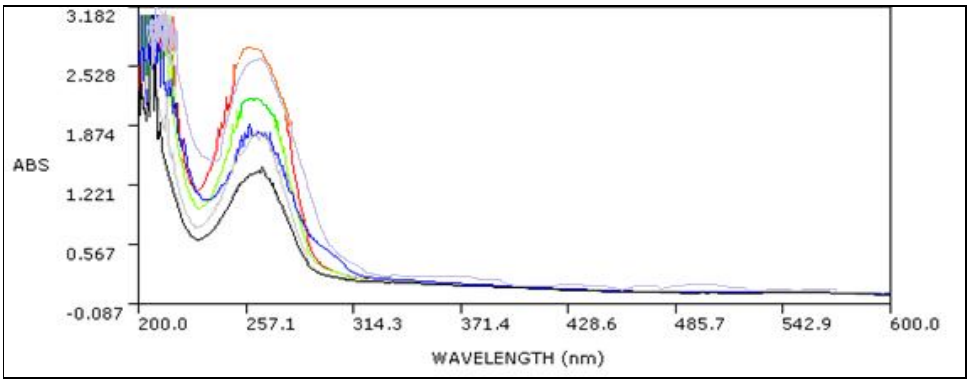


Figure 1: Absorption spectra of Fluorouracilin different α-CD concentration

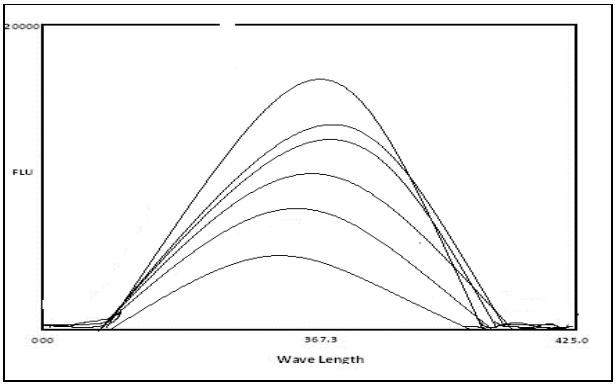


Figure 2: Fluoroescence spectra of Fluorouracil in different α-CD concentration

Table1,fig 1 & 2 shows the absorption and fluorescence spectra of fluorouracil solutions containing various concentrations of α-CD. The absorption maxima of fluorouracil appear at 265.6nm.Upon increasing the concentration of of α-CD the absorption maxima is blue shifted from 265.6nm - 246.4nm.The absorption intensities are regularly increased with increasing concentration of α-CD.Fluorescence spectra appear at 385nm.As the concentration of α-CD increases the fluorescence spectra is red shifted from 385nm - 413nm.The association constant (K) for the formation of an inclusion complex has been determined by analyzing

the changes in the absorption and fluorescence maxima with the α-CD concentration. The association constant and stoichiometric ratios of the inclusion complex of fluorouracil with α-CD can be determined by using the Benesi-Hilde brand relation.
The equations for 1:1 complexes are given below

- Absorption
  1/A-Ao =1/A-Ao+1/K (A'-Ao) [α-CD]
- Fluorescence
  1/I-Io = 1/I-Io+1/K (I'-Io) [α-CD]

In the above equation,
Ao/Io - intensity of absorption/Fluorescence of Fluorouracil without α-CD

- A/I -Absorption / Fluorescence intensity with a particular concentration of α-CD
- A'/I'- Absorption / Fluorescence intensity at the maximum concentration of α-CD used.
- K – Association constant

Linearity is obtained in the plot of 1/A-Ao or 1/I-Io verses 1/ [α-CD] for 1:1 complex.The association constant K was calculated from the slope of Benesi – Hilde brand plot using the equation [(10)].

- K = 1/slope (A'-Ao) for absorbance
- K =1/ slope (I'-Io) for fluorescence

Good linear correlations were obtained confirming the formation of 1:1inclusion complexes. The association constant K is calculated from the graph was found to be K= 382.40 M^{-1} for absorbance 148.62M^{-1} for fluorescence. The higher association constant for absorbance shows that the drug molecule completely included in to the α-CD cavity. Since the size of fluorouracil is smaller than α-CD cavity size, the drug is completely included in the cavity. Therefore the absorbance is increased.
The apparent stability constant of the complex was calculated from the phase solubility diagrams using the following equation.

- K1:1 = slope/so (1-Slope)

The slope is obtained from the initial straight line portion of the plot of concentration of fluorouracil against α-CD concentration.AL type diagram was obtained. The shape of the solubity curve indicates that a 1:1 molar ratio is most probable for the inclusion complex formed. The stability constant of fluorouracil is 125.2 M^{-1}.

5. Conclusion

The absorption maxima of fluorouracil appear at 265.6nm.Upon increasing the concentration of α-CD the absorption is blue shifted. On increasing the concentration of α-CD the fluorescence spectra is red shifted. This result indicates that fluorouracil is entrapped in the α-CD to form fluorouracil: α-CD inclusion complex..The association constant K was found to be K=382.40M^{-1} for absorption. The stability constant of fluorouracil is 125.2 M^{-1}.Addition of α-CD enhances the solubility of fluorouracil an anticancer drug. The solubility is high for the 1:1 inclusion complex.

6. References

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